Functioning of a metabolic flux sensor in Escherichia coli

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Supporting Information

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SI Text 1: Model Describing System Without Feedforward Activation

The mathematical model containing two metabolites (X and Y) and two enzymatic reactions (E1 and E2) can be described by a set of two ordinary differential equations:

\[
\frac{dX}{dt} = v - v_{E1}
\]

\[
\frac{dY}{dt} = v_{E1} - v_{E2},
\]

where \(v\) denotes the influx (input of the system) and \(v_{E1}\) and \(v_{E2}\) denote the reaction rates of \(E1\) and \(E2\), respectively. At steady state, the differential expressions are zero, simplifying Eqs. S1 and S2 to the following:

\[
v_{E1} = v_{E2} = v.
\]

The rates through the two reactions \(v_{E1}\) and \(v_{E2}\) can be described with the following equations, assuming a reversible Michaelis-Menten-type kinetic for \(E1\) and an irreversible Michaelis-Menten-type kinetic for \(E2\):

\[
v_{E1} = v = \frac{v_{max,E1} \cdot (X - \frac{Y}{K_{m,X,E1}})}{1 + \frac{Y}{K_{m,Y,E1}}} + \frac{K_{m,X,E1} \cdot (X - Y)}{K_{m,Y,E1} + Y} + X
\]

\[
v_{E2} = v = v_{max,E2} \cdot Y + \frac{K_{m,Y,E2}}{K_{m,X,E2}} + Y
\]

\(K_{m,X,E1}, K_{m,Y,E1}, \) and \(K_{m,Y,E2}\) denote the \(K_m\) values for \(X\) and \(Y\) of \(E1\) and for \(Y\) of \(E2\), respectively. \(K_{eq}\) denotes the equilibrium constant of \(E1\), and \(v_{max,E1}\) and \(v_{max,E2}\) denote the maximal possible fluxes of \(E1\) and \(E2\), respectively.

To obtain an analytical solution of the relationship of \(X\) and the flux \(v\), Eqs. S4 and S5 can be rearranged for \(X\) and \(Y\), respectively. Replacing \(Y\) in Eq. S4 (and assuming that \(K_{m,Y,E1}\) to reduce the number of parameters), one obtains the following:

\[
X = \frac{v \cdot K_{m,X,E1}}{v_{max,E1}} + \frac{v \cdot K_{m,Y,E2} \cdot v_{max,E2}}{K_{eq} \cdot (v_{max,E1} - v) \cdot (v_{max,E2} - v)}
\]

\[
+ \frac{v^2 \cdot K_{m,Y,E2}}{(v_{max,E1} - v) \cdot (v_{max,E2} - v)}.
\]

For \(v << v_{max,E1}\) and \(v << v_{max,E2}\) (which is equivalent to an influx that is much lower than the maximal possible fluxes for \(E1\) and \(E2\)), this equation simplifies to the following:

\[
X = \frac{v \cdot K_{m,X,E1}}{v_{max,E1}} + \frac{v \cdot K_{m,Y,E2}}{K_{eq} \cdot v_{max,E2}}
\]

This equation describes the analytical solution for the relationship of \(X\) and flux \(v\).

SI Text 2: Model Describing System with Feedforward Activation of \(E2\) by \(X\)

Here, we use the same ordinary differential equations as above. In contrast to the previous model, we use a Monod–Wyman–Changeux (MWC) kinetic for \(E2\) in accordance to previous studies on pyruvate kinase I (PYK I) (1–3):

\[
v_{E2} = v = \frac{v_{max,E2} \cdot Y}{K_{eq} \cdot (X + Y) + (1 + \frac{K_{m,Y,E2}}{K_{m,X,E2}}) \cdot (1 + \frac{X}{K_{m,X,E2}})}^{v-1}.
\]

\(L, n,\) and \(K_{eq} \cdot X \cdot E2\) in Eq. S8 denote allosteric equilibrium constant, cooperativity constant, and affinity constant of \(X\) for \(E2\), respectively. We chose values for \(L\) and \(n\) in accordance to parameter values that were obtained for PYK I in previous studies: PYK I is inactive in absence of its allosteric activator FBP (1, 4), which corresponds to \(L >> 1\), and several studies have determined \(n\) to be equal to 4 (1, 5). Because \(n > 1\), it is not possible to derive an analytical solution for the relationship of \(X\) and flux \(v\), and thus we solve this model equation as specified in the main text.

Abundance of intracellular FBP as a function of IPTG concentration (used as a proxy for pykF abundance) in glucose batch cultures of a pykF mutant strain bearing an IPTG-inducible PYK I expression plasmid, relative to the FBP concentration at 1 mM IPTG. Batch cultivations were performed in 500-mL shake flasks containing 30 mL of M9 glucose medium (5 g/L glucose) as described in the main text. Cultures were harvested in midexponential growth phase using fast filtration and extracted in hot ethanol as described previously (1). Extracts were then analyzed by LC-MS/MS as described in the main text.

Intracellular concentrations of enzymes of lower glycolysis at different glycolytic fluxes. Whereas the data from the four lower flux values stem from glucose-limited chemostat cultures, the highest flux data point was obtained from a glucose batch culture. Enzyme copy numbers were determined by targeted MS analysis and heavy reference peptides (1) and then converted to concentrations using the cell volumes measured in ref. 2.

